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EFFECT OF MOTOR PATTERNS ON WATER-SOLUBLE AND MEMBRANE PROTEINS AND CHOLINESTERASE ACTIVITY IN SUBCELLULAR FRACTIONS OF RAT BRAIN TISSUE

By

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At present extensive factual material has been accumulated that indicates /20** the changes in the exchange of macromolecules in the nerve tissue in different motor patterns of experimental animals [1-13]. However these works cover, as a rule, analysis of short-term muscle load.

Our task was to compare the effects of long hyperfunction, as well as prolonged hypofunction of the motor analyzer on the electrophoretic properties and activity of the acetyl- and butyrylcholinesterases in different subcellular fractions of the cerebral tissue.

Materials and Methods

Experiments were conducted on male rats of the Wistar line 60 days old. The control animals were kept during the entire experiment in standard vivarium conditions. Rats of the second group were trained every 6 days out of 7 on a moving track for about 1 h per day. The entire experiment lasted 380 days. During this time the animals ran a total of 170 km [14]. Rats of the third group were kept for all 380 days in individual cupola-shaped cages that restricted movement, but did not lead to immobilization; the weight of the animals at the end of the experiment did not differ from the weight of the control rats [14].

In 380 days after the beginning of the experiment the rats of all three

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**Numbers in margin indicate pagination in original foreign text.

groups were decapitated under light ether. The brain was quickly removed, the frontal section of the large hemispheres was excised that contains the motor zone of the cortex, it was homogenized for production of subcellular fractions and determination of cholinesterase activity, and was also treated with distilled water or Triton X-100 to extract the proteins and for their subsequent electrophoretic separation.

The 10% homogenate of brain tissue in 0.32 M of sucrose obtained in a glass homogenizer with teflon pestle was centrifuged for 15 min (800 g) in a centrifuge with cooling. The supernatant fluid was again centrifuged for 1 h (16,500 g), the obtained precipitate was resuspended in 60 ml of 0.32 M of sucrose, and the supernatant fluid was preserved for subsequent obtaining of a fraction of microsomes. The resuspended precipitate was applied to a two-stage (0.8 and 1.2 M) gradient of density of sucrose and was centrifuged for 2 h (60,000 g) in a rotary bucket (volume of test tube 60 ml).

As a result three bands were obtained: interphase 0.32-0.8 M of sucrose (fraction of myelin), 0.8-1.2 M of sucrose (fraction of synaptosomes) and precipitate on the bottom of the test tube (purified mitochondria). The myelin and synaptosomal fractions were diluted with a fourfold volume of 0.32 M of /21
sucrose and for their precipitation were centrifuged for 15 min at 120,000 g. The microsomal fraction was obtained from the supernatant fluid after precipitation of the unpurified mitochondria (16,500 g) by centrifuging for 1 h (150,000 g). As a result, the following fractions were isolated: myelin, synaptosome, purified mitochondria and microsome.

To extract the water-soluble proteins a 60% suspension was made of each subcellular fraction of cerebral tissue in distilled water and it was exposed to three-fold freezing-unfreezing at -25°C, after which it was centrifuged for 15 min. (30,000 g) on a preparation ultracentrifuge with cooling MSE-65. For extraction of the membrane proteins a 20% suspension of each fraction in 0.2% Triton X-100 was incubated for 15 min. in the cold and centrifuged for 15 min (30,000 g, MSE-65). The supernatant liquid was used for electrophoretic separation of proteins.

Disk-electrophoresis in a 7% polyacrylamide gel was carried out for 2.5 h at room temperature and current strength 2 mA on a sample in tris-glycine buffer

pH 9.5. In the beginning no less than 200 μg of protein was applied. The gel was stained for 1 h with 0.1% kumassi bright blue R-250 in 50% tricholoroacetic acide and washed in 7.5% acetic acid.

The acetyl- and butyrylcholinesterase activity was determined according to the method of Ellman with acetylthiocholine and butyrylthiocholine as a substrate and were expressed as the number of micromoles of substrate hydrolyzed during incubation for 60 min. at 25°C, in calculation for 1 mg of protein of the fraction; it is shown that under these conditions during the entire time of incubation the rate of the reaction is linear [15]. The protein content was determined with the Folin-Chokalte reagent according to the method of Lowry et al. [16].

Results and Discussion

As is apparent from fig. 1 on the electrophoregrams of proteins isolated from all the subcellular fractions, in the control animals one can isolate three zones that are distinguished by electrophoretic mobility: zone near the start, intermediate zone and zone of proteins with high electrophoretic mobility. The separation of individual proteins in these zones was pronounced better for the fraction of synaptosomes.

Prolonged muscle load results in an increase in the number of water-soluble proteins in the zone near the start in the synaptosomes and to a decrease in their number in the mitochondria. In the synaptosomes, in addition, the quantity of proteins is also increased in the intermediate zone (fig. 2). In the remaining subcellular fractions the number of water-soluble proteins is not significantly altered. The motor load does not have a visible effect on the quantity of membrane (soluble in Triton X-100) proteins.

Prolonged hypokinesia is accompanied by a reduction in the content of membrane proteins in the intermediate zone near the start in the synaptosomal fraction (fig. 3). In the mitochondria the number of membrane proteins is decreased only in the zone near the start. The number of water-soluble proteins in all the subcellular fractions is almost not altered.

Both comparable motor patterns do not affect the quantity and electrophoretic spectrum of the microsomal proteins.

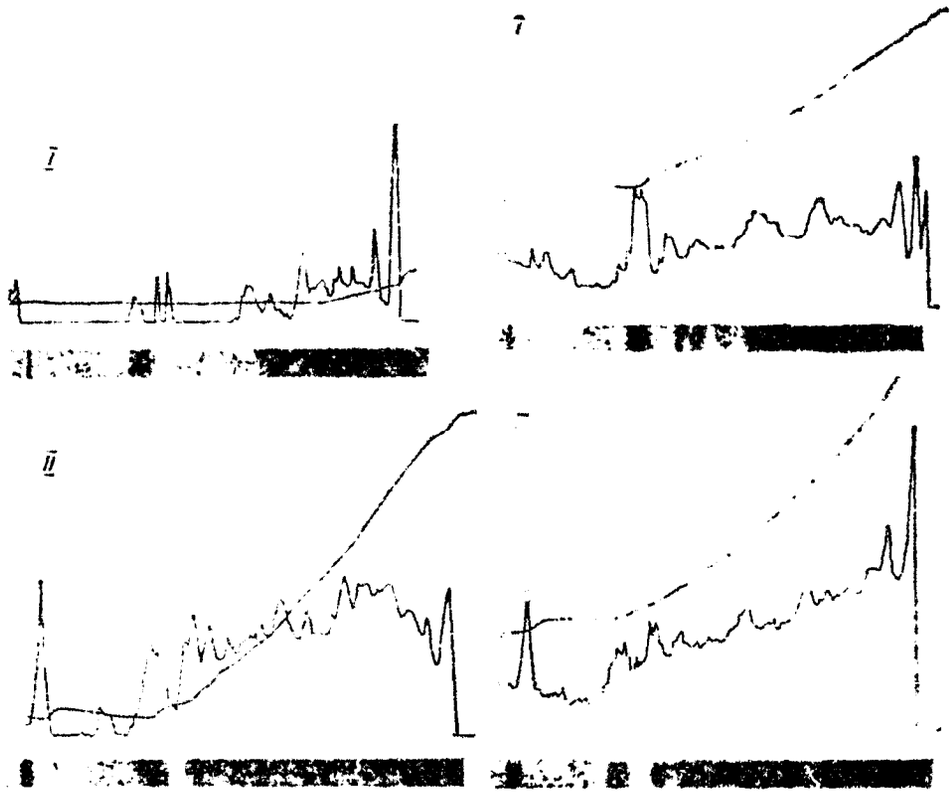

Figure 1. Electrophoregram and Densitogram of Water-Soluble (I) and Triton-Soluble (II) Proteins of Synaptosomes in Brain of Control Rats

Figure 2. Electrophoregram and Densitogram of Water-Soluble (I) and Triton-Soluble (II) Proteins of Synaptosomes in Brain of Rats after Motor Load

After determination of the cholinesterase activity it was found that in the general homogenate of brain tissue not statistically-reliable changes were found in either the acetylcholinesterase or the butyrylcholinesterase activity either with the increased or decreased motor activity of the animals (see table). Analysis of the individual subcellular fractions demonstrated the reliable ($d < 0.05$) increase in the acetylcholinesterase activity by 50% in the synaptosomes and by 45% in the mitochondria. Butyrylcholinesterase activity remains practically unchanged. Hypokinesia leads to a decrease in the acetylcholinesterase activity by 34% in the synaptosomes in the absence of reliable changes in the mitochondria. The same pattern is observed in the relationship of butyrylcholinesterase activity. In the myelin both motor patterns induce the same increase in activity of the acetylcholinesterase; the activity of butyrylcholinesterase also is not altered (see table).

EFFECT OF DIFFERENT MOTOR PATTERNS ON ACTIVITY OF ACETYL- AND BUTYRYLCHOLINESTERASE IN DIFFERENT SUBCELLULAR FRACTIONS OF RAT BRAIN

Fraction of brain tissue	Control	Motor load	Hypokinesia
	Acetylcholinesterase		
Total homogenate	23.7±4.2	20.9±2.4	25.6±3.1
Myelin	23.6±3.6	40.7±4.1*	46.7±6.2*
Synaptosomes	21.2±2.3	31.8±2.7*	14.1±2.2
Purified mitochondria	6.9±0.7	10.0±0.7*	5.7±0.6
	Butyrylcholinesterase		
Total homogenate	3.3±0.4	2.9±0.3	3.5±0.3
Myelin	11.0±0.5	9.6±0.6	10.2±0.5
Synaptosomes	5.2±0.3	4.7±0.4	3.1±0.4*
Purified mitochondria	3.6±0.4	3.0±0.4	3.6±0.4

Note: Asterisks designate statistically reliable ($d<0.05$) difference from the control; $n=5$.

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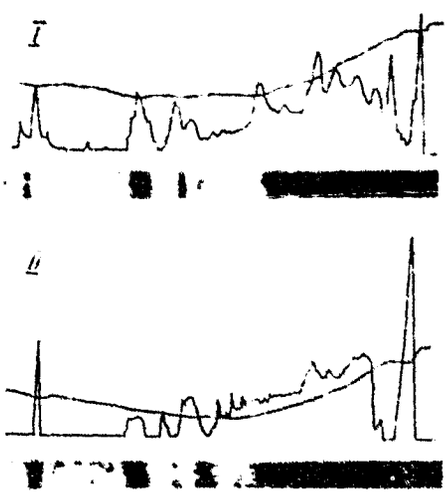

Figure 3. Electrophoregram and Densitogram of Water-Soluble (I) and Triton-Soluble (II) Proteins of Synaptosomes of Rat Brain after Hypokinesia

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Thus, the electrophoretic properties of the proteins in the brain and the activity of cholinesterase are altered mainly in the synaptosomes, to a lesser degree--in the mitochondria. It is important that very drastic changes in the enzymatic activity in the synaptosomes are not found during their analysis in the brain tissue homogenate.

Changes in the studied enzymatic activity can be a result of the increase or decrease in the intensity of synthesis of new molecules of the enzymatic protein or activation or inhibition of the enzyme without changes in the intensity of its biosynthesis. Changes in the acetylcholinesterase activity clearly correlate with the unidirectional changes in the quantity of proteins on the electrophoregram in the zone near the start, in which, as was previously shown [17] all three iso-enzymes of acetylcholinesterase are located.

Shifts in the latter activity were also noted in the purified mitochondria (see table). This can be linked to the non-mediator role of acetylcholine that in the mitochondria apparently participates in the regulation of the permeability of the mitochondrial membranes and biosynthesis of certain proteins [18,19].

Judging from the amount of the electrophoretic mobility, among the proteins that we detected, there are specific proteins of the brain: in the intermediate zone--the complex 14-3-2, and in the zone that is farthest from the start, the protein S-100 [20-22]. In hyper- and hypokinesia the number of proteins is altered in the intermediate zone, while in the zone the farthest from the start there are no changes. If one adopts the viewpoint on the primary localization of protein 14-3-2 in the neurons, and protein S-100 in the glia [10,20-23] one can hypothesize that the functionally-governed reconstruction of metabolism in the central section of the motor analyzer is localized to a greater degree in the neurons than in the cells of the neuroglia. This corresponds to our data previously obtained in experiments with short-term muscle load [2,3,5,24].

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